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L3: Entry 51 of 51 File: USPT Jan 17, 1995

DOCUMENT-IDENTIFIER: US 5382657 A
TITLE: Peg-interferon conjugates

Abstract Text (1):
Substituted PEG-interferon conjugates of formulae IA and IB where PEG is linked by means of activated linking reagents of formulae IIA, IIB, or IIB-1 to an amino group in the interferon, and activated linking reagents of formulae IIA, IIB, or IIB-1. The conjugates are not readily susceptible to in vivo hydrolytic cleavage, have enhanced in vivo half life, and reduce the immunogenicity of the interferon while maintaining biological activity.

Detailed Description Text (5):
In accordance with this invention, x, y, and z can be selected from any combination of numbers such that the resulting conjugate contains at least a portion of the biological activity of the IFN which forms the conjugate. It is apparent that the sum of x, y, and z, and m is inversely proportional to the amount of biological activity of the IFN which is retained by the conjugate. The numerical value of x, y, and z represent the number of glycol units in the polyglycol which form the conjugate. The term m represents the number of free or accessible amino groups contained by the IFN which can react with the activated PEG mixture. The higher the value of m, and x, y, and z, the higher the molecular weight of the conjugate. In accordance with this invention x, y and z are any number so that molecular weight of the conjugate, excluding the weight of the protein, is between about 300 to about 30,000 daltons. Preferably for IFN, m is a number from 1 through 3. A highly preferred embodiment is a monoPEGylated conjugate where m is 1, produced by conditions such that a high yield is obtained of IFN conjugate composed of IFN where only one free amino group has reacted with the PEG reagent of formula II-A, or II-B, or II-B1. In accordance with a preferred embodiment where m is 1, x, y, and z are any number so that the glycol which forms the conjugate has an average molecular weight of from about 300 to about 30,000 daltons, preferably about 1,000 to about 10,000 daltons, especially about 1,000 to about 5,000 daltons. In a particularly preferred embodiment, the molecular weight is about 2,000 daltons. In one preferred embodiment of the conjugates of formulae IA and IB, x and y are 5 to 500 and z is 0 to 4. In a particularly preferred embodiment, the glycol used is a mixture of glycols wherein x is between 10 to 100, y is between 1 to 10 and z is 0.

Detailed Description Text (24):
From a suspension of 1.5 g MPEG (methoxypolyethylene glycol) (m.w. -5000) in 80 ml of dry toluene was distilled 50 ml of solvent. The solution was then cooled and 30.5 mg of di-2-pyridylcarbonate added. The resulting mixture was then refluxed for 24 hr. The solution was then cooled and the resulting precipitate filtered and washed with a small volume of toluene followed by diethyl ether. The solid was then dried under high vacuum to give 0.6 g of alpha, alpha'-oxomethylene bis(omega-methoxypoly(oxy-1,2-ethanediyl) SRU 111 as a white powder. PEG-modified interferon was prepared by method 1 described below.

Detailed Description Text (25):

Preparation of PEG-modified Interferon-alpha

Detailed Description Text (28):
The solution containing PEG-interferon, quenched reagent and unmodified interferon was diluted four-fold with 40 mM ammonium acetate, pH 4.5, and loaded onto a CM-cellulose column (Whatman CM-52, approximately 0.5 ml resin per mg protein). After washing the column by 5 volumes of 40 mM ammonium acetate, pH 4.5, PEG-interferon and unmodified interferon were eluted using a linear sodium chloride gradient (0-0.5M) in the 40 mM ammonium acetate pH 4.5. Fractions containing protein were identified by absorbance at 280 nm, and PEG-interferon containing fractions were identified by SDS-PAGE.

Detailed Description Text (29):
PEG-interferon was further purified by size exclusion-gel filtration chromatography on a column containing Sephacryl S-200 resin (Pharmacia LKB). Fractions eluted from the column were analyzed by SDS-PAGE and the peak material containing PEG-interferon pooled.

Detailed Description Text (32):
By the procedure described in Example 1, MPEG (m.w. 1300) was converted to alpha,alpha-oxomethylene bis[omega-methoxypoly(oxy-1,2-ethanediyl) SRU 28.3, and PEG-modified interferon was prepared using this reagent by method 1 described in Example 1.

Detailed Description Text (37):
PEG-modified interferon was prepared using this reagent by method 1 described in Example 1.

Detailed Description Text (42):
PEG-modified interferon was prepared using this reagent by method 1 described in Example 1.

Detailed Description Text (45):
From a solution of 0.5 g of alpha-2-[2-(hydroxypropoxy)propyl]-omega-methoxypoly (oxy-1,2-ethanediyl) SRU 64.7 in 40 ml of dry CH.sub.2 Cl.sub.2 was distilled 15 ml of solvent. To the solution was then added 108 mg of di-2-pyridyl carbonate, 4 mg of DMAP and several beads of 4 A molecular sieve. The mixture was then stirred overnight, filtered and the solvent was then removed under reduced pressure. The residue was purified by means of size exclusion chromatograph. PEG-modified interferon was prepared using this reagent by method 1 described in Example 1.

Detailed Description Text (48):
By the procedure described in Example 5, alpha-2-[2-(hydroxypropoxy)propyl]-omega-methoxypoly(oxy-1,2-ethanediyl) SRU 110, was converted to alpha-methyl-omega-[2-[2-[[(2-pyridinyloxy)carbonyl]oxy]propoxy]propoxy]po ly(oxy-1,2-ethanediyl) SRU 110. PEG-modified interferon was prepared using this reagent by method 1 described in Example 1.

Detailed Description Text (51):
From a solution of 1 g of Jeffamine M-2070 (Texaco Chemical Co.) in 40 ml of dry CH.sub.2 Cl.sub.2 was distilled 15 ml of solvent. The solution was cooled to 0.degree. C. and 215 mg of di-2-pyridyl carbonate was added. The resulting solution was stirred for an additional 4 hr at 0.degree. C. after which time the solvent was removed under reduced pressure. The residue was then purified by means of two size exclusion columns attached in sequence (500 .ANG. and 1000 .ANG.). The product shows two bands in the UV at 232 nm and 310 nm. PEG-modified interferon was prepared using this reagent by method 2 described in Example 1.

Detailed Description Text (54):
By the procedure described in Example 7, 1 g of Jeffamine M-2070 was reacted with

bis(3-methyl-2-pyridyl)carbonate to give methyloxirane, polymer with oxirane, [2-[[(3-methyl-2-pyridinyloxy) carbonyl]amino]propyl methyl ether (MO/O=10/32). PEG-modified interferon was prepared using this reagent by method 1 described in Example 1.

Detailed Description Text (57):
By the procedure described in Example 7, 0.6 g of Jeffamine M-1000 (Texaco Chemical Co.) was reacted with 155.6 mg of di-2-pyridyl carbonate to give methyloxirane, polymer with oxirane, [2[[(2-pyridinyloxy)carbonyl]amino]propyl methyl ether, block (MO/O=1.6/18.6). PEG-modified interferon was prepared using this reagent by method 1 described in Example 1.

Detailed Description Text (58):
Antiviral activity of interferon: Antiviral activity of interferon and PEG-modified interferon was determined (Rubenstein, et al., (1981) J. Virol. 37: 755-758; Familletti, et al., (1981) Methods Enzymol. 78: 387-394). All assays were standardized relative to control. The interferon standard used in the assay had specific activity of 2.times.10.sup.8 units per mg of protein.

Detailed Description Text (59):
Conditions used for modification of interferon were based on optimized protocols as described. PEG-modification was analyzed by SDS-PAGE for conversion of interferon to monoPEG-interferon over various times of incubation (chemical reactivity), and for distribution into different species of PEG-interferon conjugates (site selectivity). In SDS-PAGE, PEG-modified species were observed as slower migrating bands on the gel. Both monoPEG and diPEG-interferons were produced in sufficient yield so that these species could be purified from the reaction mixtures by hydrophobic interaction chromatography. Purified PEG-interferons were tested for antiviral activity and compared with unmodified interferon-.alpha.2a standards. The molecular weights of the polymers used as well as the antiviral activity of some of the pegylated derivatives are described in Table 1.

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L3: Entry 49 of 51 File: USPT Nov 16, 1999

DOCUMENT-IDENTIFIER: US 5985265 A
TITLE: N-terminally chemically modified protein compositions and methods

Detailed Description Text (25):
The water soluble polymer may be of the type described above, and should have a single reactive aldehyde for coupling to the protein. For polyethylene glycol, use of PEG 6000 for coupling to G-CSF and PEG 12000 for consensus interferon are described below. It is noted, that for G-CSF, PEG 12000, 20000 and 25000 have also been used successfully in the present methods. Polyethylene glycol propionaldenhyde (see, e.g., U.S. Pat. No. 5,252,714) is advantageous for its stability in water.

Detailed Description Text (31):
In yet another aspect of the present invention, provided are pharmaceutical compositions of the above. Such pharmaceutical compositions may be for administration for injection, or for oral, pulmonary, nasal or other forms of administration. In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of monopolymer/protein conjugate products of the invention together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present N-terminally chemically modified proteins. See, e.g., Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, Pa. 18042) pages 1435-1712 which are herein incorporated by reference.

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L3: Entry 48 of 51 File: USPT Dec 11, 2001

DOCUMENT-IDENTIFIER: US 6329175 B1
TITLE: Interferon-.epsilon.

Detailed Description Text (165):
PEGylation by acylation typically requires reacting an active ester derivative of PEG with an interferon-.epsilon. polypeptide. An example of an activated PEG ester is PEG esterifed to N-hydroxysuccinimide. As used herein, the term "acylation" includes the following types of linkages between interferon-.epsilon. and a water soluble polymer: amide, carbamate, urethane, and the like. Methods for preparing PEGylated interferon-.epsilon. by acylation will typically comprise the steps of (a) reacting an interferon-.epsilon. polypeptide with PEG (such as a reactive ester of an aldehyde derivative of PEG) under conditions whereby one or more PEG groups attach to interferon-.epsilon., and (b) obtaining the reaction product(s). Generally, the optimal reaction conditions for acylation reactions will be determined based upon known parameters and desired results. For example, the larger the ratio of PEG: interferon-.epsilon., the greater the percentage of polyPEGylated interferon-.epsilon. product.

Detailed Description Text (167):
PEGylation by alkylation generally involves reacting a terminal aldehyde derivative of PEG with interferon-.epsilon. in the presence of a reducing agent. PEG groups are preferably attached to the polypeptide via a --CH.sub.2 --NH group.

Detailed Description Text (298):
Administration of a molecule having interferon-.epsilon. activity to a subject can be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, by perfusion through a regional catheter, or by direct intralesional injection. When administering therapeutic proteins by injection, the administration may be by continuous infusion or by single or multiple boluses. Alternatively, interferon-.epsilon. can be administered as a controlled release formulation. For example, Cleland and Jones, Pharm. Res. 13:1464 (1996), describe a method for producing interferon-.gamma. encapsulated in polylactic-coglycolic microspheres.

L3: Entry 33 of 51 File: PGPB Feb 26, 2004

DOCUMENT-IDENTIFIER: US 20040037809 A1
TITLE: Compositions and methods for enhanced mucosal delivery of interferon beta

Summary of Invention Paragraph:
[0322] Additional absorption-promoting polymers for use within the invention are those classified as dextrans, dextrins, and from the class of materials classified as natural gums and resins, or from the class of natural polymers such as processed collagen, chitin, chitosan, pullalan, zooglan, alginates and modified alginates such as "Kelcoloid" (a polypropylene glycol modified alginate) gellan gums such as "Kelocogel", Xanathan gums such as "Keltrol", estastin, alpha hydroxy butyrate and its copolymers, hyaluronic acid and its derivatives, polylactic and glycolic acids.

Summary of Invention Paragraph:
[0431] The biologically active agent may be dispersed in a base or vehicle, which may comprise a hydrophilic compound having a capacity to disperse the active agent and any desired additives. The base may be selected from a wide range of suitable carriers, including but not limited to, copolymers of polycarboxylic acids or salts thereof, carboxylic anhydrides (e.g. maleic anhydride) with other monomers (e.g. methyl (meth)acrylate, acrylic acid, etc.), hydrophilic vinyl polymers such as polyvinyl acetate, polyvinyl alcohol, polyvinylpyrrolidone, cellulose derivatives such as hydroxymethylcellulose, hydroxypropylcellulose, etc., and natural polymers such as chitosan, collagen, sodium alginate, gelatin, hyaluronic acid, and nontoxic metal salts thereof. Often, a biodegradable polymer is selected as a base or carrier, for example, polylactic acid, poly(lactic acid-glycolic acid) copolymer, polyhydroxybutyric acid, poly(hydroxybutyric acid-glycolic acid) copolymer and mixtures thereof. Alternatively or additionally, synthetic fatty acid esters such as polyglycerin fatty acid esters, sucrose fatty acid esters, etc. can be employed as carriers. Hydrophilic polymers and other carriers can be used alone or in combination, and enhanced structural integrity can be imparted to the carrier by partial crystallization, ionic bonding, crosslinking and the like. The carrier can be provided in a variety of forms, including, fluid or viscous solutions, gels, pastes, powders, microspheres and films for direct application to the nasal mucosa. The use of a selected carrier in this context may result in promotion of absorption of the biologically active agent.

Summary of Invention Paragraph:
[0437] Exemplary polymeric materials for use in this context include, but are not limited to, polymeric matrices derived from copolymeric and homopolymeric polyesters having hydrolysable ester linkages. A number of these are known in the art to be biodegradable and to lead to degradation products having no or low toxicity. Exemplary polymers include polyglycolic acids (PGA) and polylactic acids (PLA), poly(DL-lactic acid-co-glycolic acid)(DL PLGA), poly(D-lactic acid-coglycolic acid)(D PLGA) and poly(L-lactic acid-co-glycolic acid)(L PLGA). Other useful biodegradable or bioerodable polymers include but are not limited to such polymers as poly(epsilon-caprolactone), poly(epsilon-aprolactone-CO-lacti- c acid), poly(.epsilon.-aprolactone-CO-glycolic acid), poly(beta-hydroxy butyric acid), poly (alkyl-2-cyanoacrilate), hydrogels such as poly(hydroxyethyl methacrylate), polyamides, poly(amino acids) (i.e., L-leucine, glutamic acid, L-aspartic acid and the like), poly(ester urea), poly(2-hydroxyethyl DL-aspartamide), polyacetal

polymers, polyorthoesters, polycarbonate, polymaleamides, polysaccharides and copolymers thereof. Other useful formulations include controlled-release compositions such as lactic acid-glycolic acid copolymers useful in making microcapsules and other formulations (U.S. Pat. Nos. 4,677,191 and 4,728,721), and sustained-release compositions for water-soluble peptides (U.S. Pat. No. 4,675,189).

CLAIMS:

22. The pharmaceutical composition of claim 21, wherein the sustained release-enhancing agent is polyethylene glycol (PEG) in combination with interferon-.beta..

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L3: Entry 31 of 51 File: PGPB Jun 17, 2004

DOCUMENT-IDENTIFIER: US 20040115774 A1
TITLE: Polymer-modified synthetic proteins

Summary of Invention Paragraph:
[0016] Given the inability to site-specifically modify proteins containing multiple amino acids with side-chains bearing the same or similar reactive functional groups, recent efforts have focused on the modification of the amino or carboxy terminus of proteins. Modification of the amino or carboxy terminus has relied on the ability of some chemical conjugation techniques to uniquely modify these sites (WO 90/02136 and WO 90/02135). For example, this technique was utilized for the attachment of PEG chains to the N-terminal residue of G-CSF and the chemokine IL-8 (Gaertner et al., Bioconjug. Chem. (1996) 7(1):38-44; and WO 96/41813). However, modification of the N- or C-termini typically reduces a protein's activity (See, e.g., U.S. Pat. No. 5,985,265 discussing attachment of PEG to the N-terminus and lysine side chains of G-CSF). Despite the drawbacks with modification of proteins with water-soluble polymers, PEGylation and attachment of other water-soluble polymers to proteins continues to be pursued. For example, attachment of PEG to histidine in IF-alpha has been described in U.S. Pat. Nos. 5,951,974 and 6,042,822. Attachment of PEG to lysines in alpha interferon is described in U.S. Pat. No. 5,595,732. Attachment of PEG to sugar chains of erythropoietin (EPO) and internal amino acids such as lysines (EP 0 605 963 and WO 00/32772), and the N-terminus (U.S. Pat. No. 6,077,939 and WO 00/32772) also has been described.

Detail Description Paragraph:
[0306] In a preferred embodiment, such drug delivery devices will respond to changes in the biological environment and deliver--or cease to deliver--drugs based on these changes. A range of materials have been employed to control the release of drugs and other active agents.: poly(urethanes), poly(siloxanes), poly(methyl methacrylate), poly(vinyl alcohol) for hydrophilicity and strength, poly(ethylene), poly(vinyl pyrrolidone), poly(2-hydroxy ethyl methacrylate), poly(n-vinyl pyrrolidone), poly(methyl methacrylate), poly(vinyl alcohol), poly(acrylic acid), polyacrylamide, poly(ethylene-co-vinyl acetate), poly(ethylene glycol), poly (methacrylic acid), etc. In a further preferred embodiment, biodegradeable polymers will be employed to facilitate drug delivery. Such polymers incude polylactides (PLA), polyglycolides (PGA), poly(lactide-co-glycolides) (PLGA), polyanhydrides, and polyorthoesters. Drug delivery devices, and methods for their use are described in U.S. Pat. Nos.; 6,072,041; 6,041,253; 6,018,678; 6,017,318; 6,002,961; 5,879,712; 5,849,331; 5,792,451; 5,783,212; 5,766,633; 5,759,566; 5,690,954; 5,681,811; 5,654,000; 5,641,511; 5,438,040; 4,810,499; and 4,659,558.

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